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EFFECTS OF FLOWERING STAGE AND DRYING METHOD ON THE BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *OPUNTIA STRICTA* FLOWERS AND SENSORY ACCEPTABILITY OF A CITRIC-ACID-PRETREATED INFUSION

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ABSTRACT

The growing awareness of the role of nutrition in health promotion has increased consumer interest in beverages that are both refreshing and functional. Undervalued plant resources rich in bioactive compounds present opportunities for the development of novel functional foods and beverages. *Opuntia stricta* (OS) cactus flowers contain bioactive compounds, making them promising candidates for functional beverage development. This study evaluated the effects of flowering stage and drying methods: freeze-drying, oven-drying and solar-drying on the bioactive composition and *in vitro* antioxidant activity of *Opuntia stricta* flowers. It also assessed the sensory acceptability of an infusion prepared from citric-acid-pretreated, dried flowers. Total flavonoid content ranged from 410 to 5249 mg Quercetin Equivalent (QE)/100 g dry weight (DW), total phenolic content from 540 to 1534 mg Gallic Acid Equivalent (GAE)/100 g DW, total betalain content from 21 to 358 mg/100 g DW and anthocyanin content from 1033 to 2577 mg/100 g DW. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (10 mg/mL) ranged from 4% to 81%. Flowering stage, drying method and their interaction significantly affected all measured parameters ($p < 0.05$). Freeze-drying consistently retained the highest levels of bioactive compounds and antioxidant activity across all flowering stages, whereas solar-drying resulted in the greatest losses. Anthesis flowers exhibited the highest levels of total phenolics, flavonoids, betalains and antioxidant activity, while anthocyanin content was highest at the budding stage. Despite the superior preservation achieved by freeze-drying, oven-drying was selected for product development due to its practicality, affordability and suitability for wider-scale application. The sensory analysis results showed that both 0.5% and 1% citric acid treatments improved the acceptability compared to the control, with the 1% treatment achieving the highest mean overall-liking score but was not significantly different from 0.5%. Overall, the findings demonstrate that harvesting the flowers of OS at anthesis and then oven-drying them could be a viable method for producing a beverage from this underutilized species. Pretreatment with citric acid improved the sensory acceptability, highlighting the potential of OS flowers for value addition. These findings highlight cactus flowers as promising sources of bioactive compounds for sustainable, locally relevant functional beverage development.

Key words: *Opuntia stricta*, phytochemicals, antioxidant, infusion, sensory, bioactive, drying, functional beverage.

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INTRODUCTION

Opuntia stricta of the family Cactaceae is abundantly spread in arid and semi-arid lands (ASALs) and has been found to be invasive in many parts of Kenya including Laikipia, Baringo, Samburu and Isiolo. *Opuntia stricta* is thought to have been introduced as an ornamental plant and subsequently naturalized in ASALs [1]. While the invasiveness of this plant is thought to inhibit native vegetation, change grazing behaviour and contribute to land degradation [2], its flowers may represent an underutilized resource worth exploring for food applications. Research on other cactus species has highlighted the nutritional and functional properties of their flowers and their potential use in food products [3].

Growing concern with foods that promote good health has led to an increase in demand for functional beverages, herbal infusions in particular that serve as healthy alternatives to carbonated and caffeinated beverages. Functional beverages contain phytochemicals possessing antioxidant activity and are thought to be able to reduce oxidative stress associated with many chronic diseases [4]. Recently, cactus flowers have been noted as one of the sources of bioactive compounds such as flavonoids, phenolic acids, betalains and carotenoids, several of which have been associated with antioxidant activity in experimental studies [5]. Among the major compounds contained in cactus flowers are flavonoids including quercetin, isorhamnetin and their derivatives [3].

Some studies have investigated the chemical composition and the antioxidant activities of Opuntia flowers in varying flowering stages. Such studies have indicated that the maturity stage of flowers may affect the composition and antioxidant properties of such flowers [6]. Nevertheless, there has been limited research regarding the combined effects of flowering stage and drying method on the bioactive composition and *in vitro* antioxidant activity of OS flowers. Furthermore, the effect of citric-acid pretreatment on the sensory acceptability of an infusion prepared from the dried flowers remains to be established.

Due to their high moisture content ranging between 75% and 88% [6], cactus flowers are quite perishable, thus, preservation is required for maintaining the nutritional and bioactivity of these flowers. Drying is one of the most common methods of preservation; however, different drying methods may lead to different levels of bioactivity and sensory characteristics of dried cactus flowers. Freeze-drying is considered to be the best method in terms of the retention of heat-labile bioactive compounds; however, its cost and technological requirements may be a limiting factor in many cases [7]. Oven-drying is a more practical and affordable approach, however, it results in losses of heat-labile and volatile bioactive compounds [8]. Solar-drying is a rather cheap and accessible way of drying, especially in rural areas. However, variations in temperature and humidity during the solar-drying process may lead to non-uniform drying, color changes and loss of antioxidant activity [7].

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This study evaluated the effects of flowering stage and drying method on the bioactive compounds and *in vitro* antioxidant activity of OS flowers and assessed the sensory acceptability of an infusion prepared from citric-acid-pretreated flowers. Specifically, it determined the betalain, anthocyanin, total phenolic and total flavonoid contents of the flowers, evaluated the *in vitro* antioxidant activity of their extracts and assessed the sensory acceptability of the infusion prepared from citric-acid-pretreated, dried flowers.

MATERIALS AND METHODS

Study area and experimental design

The study was conducted using OS flowers collected from naturally occurring cactus populations in the Loisukut area of Laikipia County, Kenya (0°04'60.00" N; 36°39'59.99" E) in December 2023 while a separate collection for sensory evaluation was conducted in April 2025. The site is characterized by open rangeland conditions, with plants growing under unmanaged, rain-fed conditions without irrigation, fertilization or other agronomic inputs. A completely randomized factorial design was applied, consisting of three flowering stages (budding, budding maturation and anthesis) and three drying methods (oven, solar and freeze-drying), resulting in nine treatment combinations (3 × 3). This design was used to evaluate the combined effects of developmental stage and post-harvest drying method on flower quality attributes.

In addition to that, sensory analysis was carried out to determine consumer acceptance of OS flower infusion made from anthesis flowers that underwent three different levels of citric acid pretreatment (0%, 0.5% and 1%). This sensory test was done separately from the 3 × 3 experiment on the flowering stage × drying method of the flowers.

Sampling procedure

Flowers of OS for bioactive compound analysis were collected from approximately 500 individual plants at the selected site during December 2023. Not all plants contributed flowers at all three developmental stages. A total of 1500 flowers were collected, comprising 500 flowers at the budding stage, 500 at the budding maturation stage and 500 at anthesis. Flowers were classified according to the morphological criteria described above. For each flowering stage, the 500 collected flowers were pooled to form a composite sample, which was then divided among the three drying methods: freeze-drying, oven-drying and solar-drying. An additional 900 anthesis-stage OS flowers were harvested separately in April 2025 specifically for the sensory evaluation. The fresh flowers were subjected to citric acid pretreatment, followed by oven-drying and milling. Approximately 250 g of dried flower powder was obtained for each treatment, giving a total of approximately 750 g.

Three distinct developmental stages were identified using morphological characteristics:

(i) Budding stage – Flowers were tightly closed, with the floral buds completely enclosed and uniformly green in color.

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(ii) Budding maturation stage – Flowers remained partially closed, but the petals had begun to expand, revealing a characteristic maroon-yellow coloration.

(iii) Anthesis stage – Flowers were fully open, with petals completely expanded and uniformly bright yellow in color.

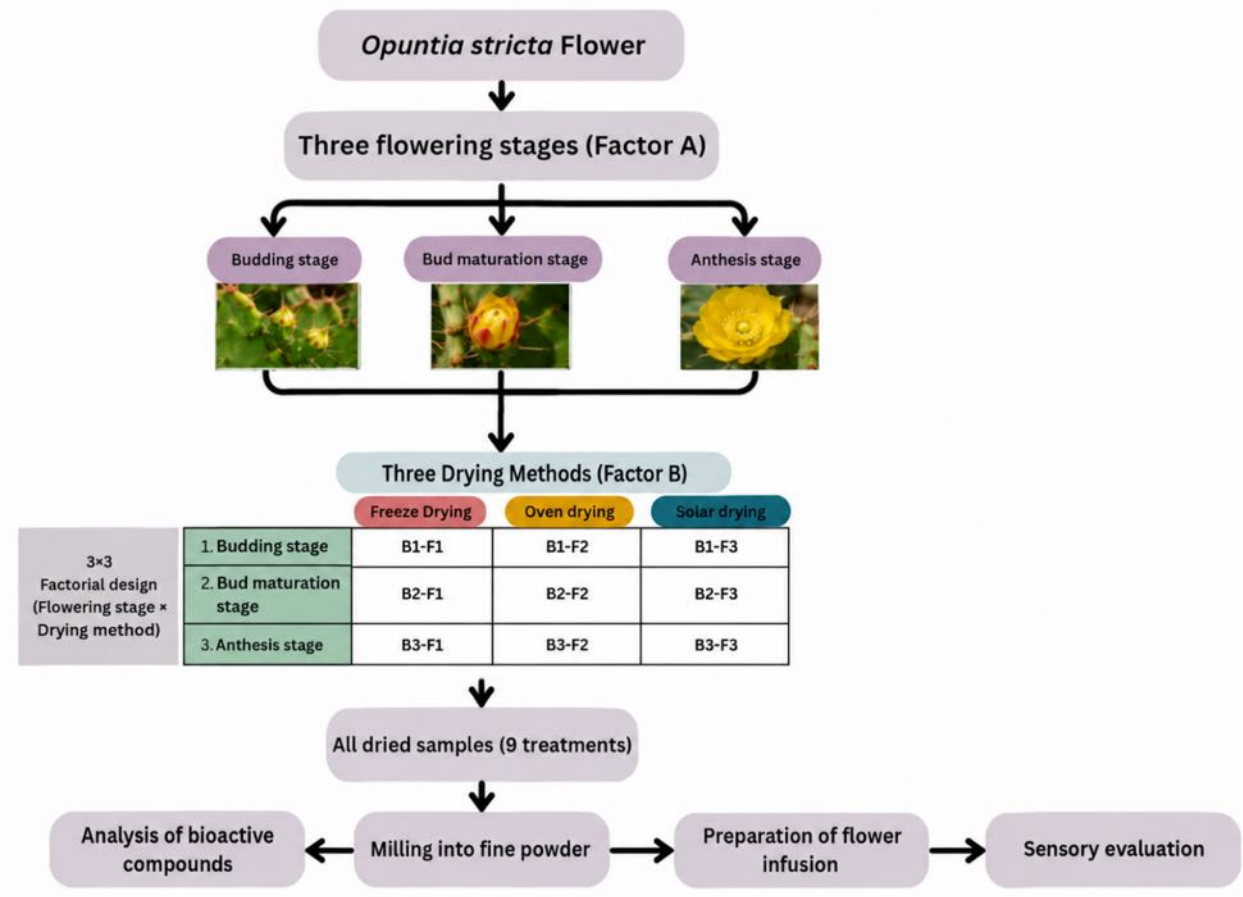


Figure 1: Experimental design and processing workflow for OS flower treatments and functional beverage development

To minimize ecological disturbance, no more than 50% of flowers were harvested from any individual plant. Each flower was excised with a small portion of the basal tissue attached to preserve structural integrity during handling and transport [9].

Sample handling and transport

Immediately after harvesting, flowers were placed in insulated cool boxes containing ice packs. Samples were transported to the Food Science Laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT) under chilled conditions for further processing. Transport time was minimized to preserve post-harvest biochemical integrity.

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Sample preparation

Upon arrival at the laboratory, flowers were sorted to remove damaged or discolored material. External contaminants such as dust and spines were gently removed using a soft sterile brush. Samples were then lightly rinsed with distilled water and blotted dry using sterile paper towels. To reduce biological variability, flowers were grouped according to developmental stage and size uniformity. Prepared samples were immediately subjected to drying treatments.

Drying treatments

Prepared samples were divided into nine experimental groups corresponding to the factorial combination of flowering stage and drying method. Each of the nine treatment samples was analyzed in triplicate, with three repeated measurements performed on the same sample to assess analytical repeatability. The drying endpoint for each method was predetermined based on fixed drying durations established in the experimental protocol: 72 h for freeze-drying, 48 h for oven drying and 72 h for solar-drying. These durations were selected to ensure adequate drying of the flower samples under the respective drying conditions [7]. Following drying by freeze-drying (FD), oven-drying (OD) and solar-drying (SD), the moisture content of the dried flower samples was determined gravimetrically using the oven-drying method (TD 384KN, Tokyo, Japan). The final moisture content was calculated from the mass loss during oven-drying and expressed as a percentage which ranged between 5–12%.

Freeze-drying

Samples were pre-frozen at temperatures below −18 °C for 72 hours to ensure complete solidification of free water. Freeze-drying was then conducted using a laboratory freeze dryer (Model FDL-10N-50-TD-MM, Israel) operated at −54 °C and 1 kPa for 72 hours.

Oven-drying

Oven-drying was performed in a forced-air convection oven (TD 384KN, Tokyo, Japan) at 60 °C for 48 hours under controlled air circulation and relative humidity maintained at approximately 25%.

Solar-drying

Solar-drying was conducted using a greenhouse-type solar dryer at JKUAT's BED facility. Drying lasted 72 hours, with daytime temperatures ranging from 22–28 °C and nighttime temperatures from 16–18 °C. Relative humidity (50–70%) was continuously monitored using a HUATO data logger. The system was naturally ventilated to enhance moisture removal and prevent microbial growth.

Post-drying processing and storage

After drying, all samples were milled into a fine powder using a laboratory grinder under dry conditions to prevent moisture uptake. The powdered samples were sealed in airtight, labeled polyethylene zip-lock bags and stored in a dark, dry environment until further analysis to minimize photo-oxidation and hygroscopic degradation.

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Analysis of Antioxidants

Preparation of Opuntia stricta flower extracts

Samples for the analysis of phytochemicals in OS flowers were prepared using the maceration method [10]. A 0.1 g portion of dried cactus flower samples was weighed into 100 mL centrifuge bottles and 25 mL of methanol was added. The bottles were sealed, wrapped in aluminum foil to prevent light exposure and shaken for 3 h at 200 rpm using a mechanical shaker (IKA Labortechnik KS250 Basic, Germany). Extraction was continued for 72 h in the dark.

After extraction, the samples were filtered using Whatman filter paper No. 4 and the filtrate volume was adjusted to 25 mL. The extracts were centrifuged at 2500 rpm using a centrifuge (Hettich Universal 16A, Germany) at room temperature. The resulting supernatant was collected and used for the determination of total phenols, total flavonoids, anthocyanins and betalains.

Determination of total flavonoid content

Total flavonoid content was determined using the colorimetric method described by Maciel [11]. A 0.5 mL aliquot of extract was transferred into test tubes containing 4 mL distilled water. After 5 min, 0.3 mL of 5% sodium nitrite was added and vortexed, then allowed to stand for 3 min. Subsequently, 0.3 mL of 10% aluminum chloride was added and after 2 min, 2 mL of 1 M sodium hydroxide was introduced. The final volume was adjusted to 10 mL with distilled water.

The mixture was incubated at room temperature for 30 min and absorbance was measured at 415 nm using a UV–VIS spectrophotometer (Shimadzu UV-VIS 1601 PC, Tokyo, Japan). Quercetin standards (100–1000 µg/mL) were used to generate a calibration curve. Total flavonoid content was expressed as mg quercetin equivalents per 100 g of DW.

Determination of total polyphenol content

The Folin–Ciocalteu method, based on oxidation/reduction reactions of phenolic compounds, was used to determine total polyphenol content (TPC) according to Ainsworth and Gillespie [10]. A 0.5 mL aliquot of extract was placed in a foil-lined test tube, followed by the addition of 2 mL of 10% Folin–Ciocalteu reagent and vortexing.

After 5 min, 4 mL of 0.7 M sodium carbonate was added to prevent air oxidation of phenolic compounds. The mixture was incubated at room temperature in the dark for 2 h. Absorbance was measured at 760 nm using a UV–VIS spectrophotometer (Shimadzu UV–VIS 1601 PC, Tokyo, Japan). Gallic acid standards (5–50 µg/mL) were used for calibration.

The phenolic content was determined from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalent per 100 g DW.

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Determination of total betalain content

Total betalain content (TBC) of the cactus flower methanolic extract was determined using the method proposed by Gouws [12]. Absorbance of diluted methanolic extracts (10% v/v) was measured at 482, 532 and 600 nm using a UV–VIS spectrophotometer (Shimadzu UV–VIS 1601 PC, Tokyo, Japan).

Betacyanin and betaxanthin contents were estimated based on maximum absorbance, molecular weight and extinction coefficients of betanin and indicaxanthin using Equation (1):

$$\frac{(A_{max} - A_{600nm}) \times \text{Dilution factor} \times MW \times 10}{\varepsilon \times 1} \quad (1)$$

For betacyanin, Amax = 532 nm, MW = 550 g/mol, ε = 60,000 L mol^{-1} cm^{-1} (betanin equivalent). For betaxanthin, Amax = 482 nm, MW = 308 g/mol, ε = 43,000 L mol^{-1} cm^{-1} (indicaxanthin equivalent) (Gouws et al., 2019). Total betalain content was expressed as mg/100 g DW.

Determination of anthocyanin content

Anthocyanin content was determined by measuring absorbance at 510 nm and 700 nm using a UV–VIS spectrophotometer as proposed by Giusti and Wrolstad [13] with minor adjustments. The difference in absorbance was used to correct for turbidity effects. Anthocyanin content was calculated using Equation (2):

$$\frac{A \times MW \times DF \times 1000}{\epsilon \times l} \quad (2)$$

Where A = absorbance difference, MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol) or other reference anthocyanin, DF = dilution factor, ε = molar extinction coefficient of cyanidin-3-glucoside (26,900 L/mol·cm) and l = path length (1 cm).

Determination of DPPH radical scavenging activity

Antioxidant activity was evaluated through the radical scavenging activity against the free radical, DPPH, based on the method outlined by Bey [14] with minor adjustments. A 0.25 g sample of dried cactus flower was extracted in 25 mL methanol using a mechanical shaker for 3 h, followed by extraction in the dark for 72 h.

The extract was filtered (Whatman filter paper No. 4) and concentrated to approximately 20 mL using a rotary evaporator. Serial dilutions (0.625, 1.25, 2.5, 5 and 10 mg/mL) were prepared in methanol. For analysis, 1 mL of extract was mixed with 3 mL methanol and 0.5 mL of 1 mM DPPH solution in methanol. Vitamin C was used as the standard antioxidant at comparable concentrations. A blank consisted of 0.5 mL DPPH in 4 mL methanol. Absorbance was measured at 517 nm using a UV–VIS spectrophotometer.

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Radical scavenging activity was calculated using Equation 3:

$$\%\text{inhibition of DPPH} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (3)$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Sensory analysis

Sensory evaluation was conducted to determine consumer acceptance of cactus flower infusions prepared from anthesis-stage OS flowers subjected to different citric acid pretreatments (0%, 0.5% and 1%). The assessment focused on the influence of pretreatment concentration on key sensory attributes and overall acceptability. A total of 108 untrained panelists, comprising students and staff of Jomo Kenyatta University of Agriculture and Technology (JKUAT), were recruited. Participants were regular consumers of herbal or plant-based beverages. Participation was voluntary and panelists were briefed on the evaluation procedure prior to testing.

Preparation and presentation of cactus flower infusions for sensory evaluation

Fresh anthesis-stage OS flowers were harvested, ensuring they were free from visible damage, pests, or defects. The flowers were thoroughly cleaned to remove dirt, debris and other surface contaminants. Spines were removed using a combination of scraping and brushing, followed by visual inspection to ensure complete de-spining for consumer safety.

Citric acid was chosen as the pretreatment acidulant because it is a food-grade organic acid commonly used in foods and beverages for acidulation. Cleaned flowers were subjected to citric-acid pretreatment by soaking in 0.5% and 1% citric acid solutions for 15 min. A control sample (0% citric acid) was also included. The concentrations (0.5% and 1%, w/v) and soaking duration (15 min) were study-specific experimental conditions selected to investigate the effect of citric-acid pretreatment on the sensory acceptability of the flower infusion; they were not adopted as established optimum processing levels from a published study. The 0% citric acid treatment served as the control for evaluating sensory acceptability without citric-acid pretreatment.

After pretreatment, flowers were drained to remove excess solution without rinsing and then oven-dried at 60 °C until a brittle texture was achieved, corresponding to a final moisture content of approximately 8–12%. The dried material was then milled into coarse particles suitable for infusion preparation and stored in sealed zip-lock bags.

Infusions were prepared using a standardized quantity of 2.0 g of dried cactus flower material per 100 mL of hot potable water (90–95 °C) for 10 min, followed by filtration. The resulting infusions were cooled to a serving temperature of approximately 40–45 °C before evaluation.

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The three prepared infusions were served in identical, transparent, odorless cups labelled with random three-digit codes to ensure blinding. The serving order was randomized across panelists to minimize order and carry-over effects. Panelists were provided with water and instructed to rinse their mouths between samples to minimize sensory interference.

Sensory evaluation procedure

Sensory evaluation was conducted in the sensory analysis laboratory of the Department of Food Science and Technology at JKUAT under controlled conditions. The sensory attributes assessed included appearance, aroma, taste, mouthfeel, aftertaste and overall acceptability. Panelists evaluated the samples using a 5-point hedonic scale ranging from 1 ("dislike extremely") to 5 ("like extremely") and were instructed to avoid communication during evaluation and rely solely on their individual sensory perceptions.

Statistical analysis

All analyses were performed in triplicate and results expressed as mean ± standard deviation. Data for bioactive compounds and DPPH radical scavenging activity were analyzed using two-way analysis of variance (ANOVA) to determine the effects of flowering stage, drying method and their interaction. Sensory data were analyzed using one-way ANOVA. Mean separation was performed using Tukey's honestly significant difference (HSD) test at $p < 0.05$. Statistical analyses were conducted using STATA.

RESULTS AND DISCUSSION

Effect of drying methods and flowering stages on total phenolics, flavonoids and antioxidant activity

The effects of drying methods and flowering stages on total flavonoid content (TFC), total phenolic content (TPC) and DPPH radical scavenging activity are presented in Figure 2, Figure 3 and Figure 4, respectively. Total flavonoid content and total phenolic content of OS flowers were significantly influenced by flowering stage, drying method and their interaction ($p < 0.05$). Total flavonoid content values ranged from 409.81 to 5248.51 mg QE/100 g DW, while TPC ranged from 539.87 to 1534.34 mg GAE/100 g DW.

Across all treatments, freeze-drying consistently resulted in the highest measured content of both flavonoids and phenolics, followed by oven-drying, with solar-drying producing the lowest values. Anthesis-stage flowers exhibited the highest concentrations of both compounds, whereas budding-stage flowers recorded the lowest levels. The highest TFC was observed in freeze-dried anthesis flowers (5249 ± 32.4 mg QE/100 g DW), while the lowest was recorded in solar-dried budding flowers (409.8 ± 7.0 mg QE/100 g DW). Similarly, the highest TPC was recorded in freeze-dried anthesis flowers (1534 ± 1.88 mg GAE/100 g DW), whereas the lowest value occurred in solar-dried budding flowers (539.9 ± 6.9 mg GAE/100 g DW).

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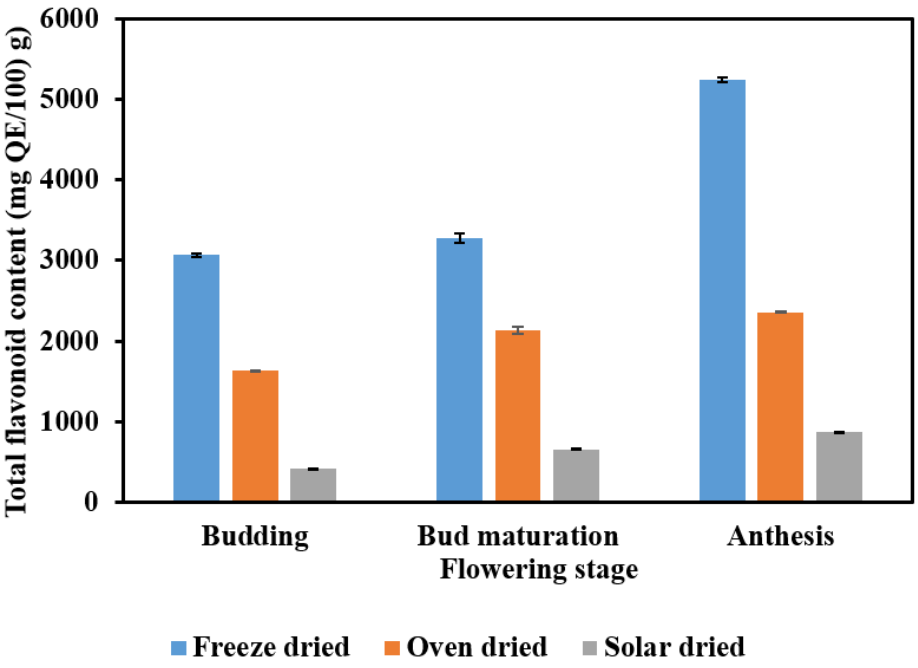


Figure 2: Effect of drying methods and flowering stages on total flavonoid content of OS flowers

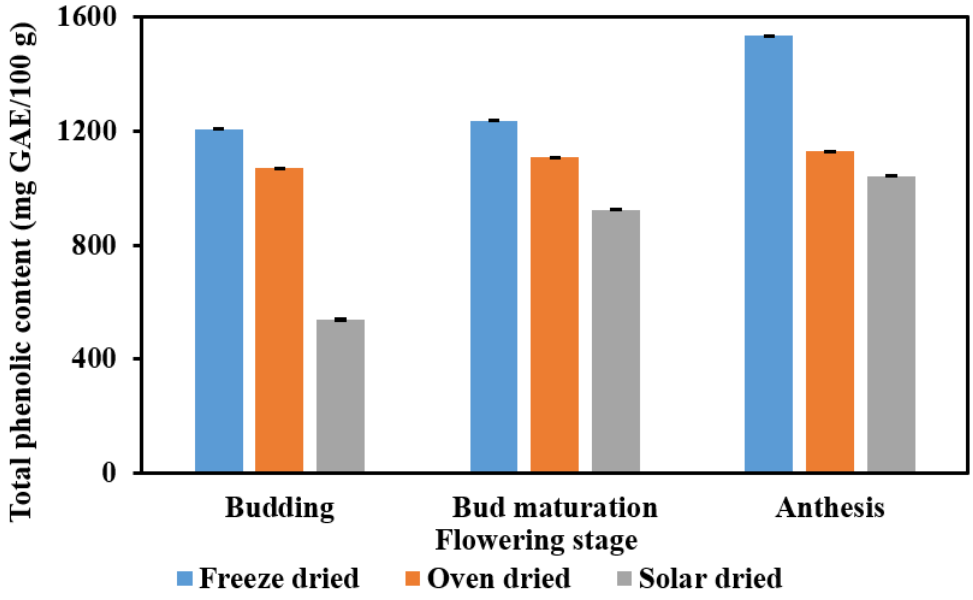


Figure 3: Effect of drying methods and flowering stages on total phenolic content of OS flowers

The excellent phytochemical retention from freeze-dried OS flowers appears consistent with observations made for other edible blooms. For freeze-dried *Hibiscus sabdariffa*,

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retention of total phenolics and flavonoids was substantially improved compared to traditional heat drying, as the low-temperature drying could prevent oxidation and enzymatic degradation [15]. Freeze-dried chamomile (*Matricaria chamomilla*) and butterfly pea (*Clitoria ternatea*) have similar observations, freeze-drying retains more bioactive compounds and antioxidant capacity than oven drying. These parallels indicate that freeze-drying appears to be generally effective in maintaining phytochemicals of different edible flower species [23]. In contrast, oven- and solar-drying expose plant tissues to prolonged heat, light and oxygen, accelerating oxidative breakdown and structural degradation of polyphenols [8].

Flower developmental stage also significantly influenced TPC and TFC, with both increasing progressively from budding to anthesis. This pattern is consistent with increased accumulation of phenolic compounds as the flowers mature, where they function in UV protection, pigmentation and antioxidant defense [18]. The significant interaction effect indicates that mature anthesis tissues, although richer in phenolics, are more vulnerable to degradation under thermal processing. Similar developmental trends have been reported for edible flowers of a variety of species, with phenolic metabolism shifting with flower maturity. Despite variation among species in accumulation pattern, studies on hibiscus and chrysanthemum have demonstrated that floral developmental stage strongly influences the concentration of phenolic compounds and antioxidant activity due to changes in secondary metabolite biosynthesis during flower maturation. Based on those observations, the present findings indicate that anthesis is a metabolically active stage of OS, associated with enhanced accumulation of antioxidant compounds [19].

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, concentration at 10 mg/mL, presented in Figure 4, ranged from 3.93 ± 0.16% to 81.35 ± 0.65% at 10 mg/mL DW and followed trends similar to TPC and TFC. The highest antioxidant activity was observed in freeze-dried anthesis flowers, while the lowest was recorded in solar-dried budding flowers. The parallel results of the DPPH activity, TPC and TFC indicate the possibility of the presence of phenolic and flavonoids responsible for the antioxidant properties of OS flowers [20]. Moreover, a positive correlation existed between phenolic molecules, flavonoids and antioxidant activity in several edible flowers, rose (*Rosa damascena*), calendula (*Calendula officinalis*), nasturtium (*Tropaeolum majus*) and lavender (*Lavandula angustifolia*) with a positive association with a significant degree of DPPH radical scavengers, with those having higher concentrations of these compounds usually showing increased DPPH radical scavenging activity. It is this, however, that seems to indicate that phenolic compounds are the dominant factors impacting on antioxidant activity in plants from various edible flower species. Accordingly, the high antioxidant activities of freeze-dried anthesis-stage OS flowers further demonstrate their potential as a valuable functional ingredient for the development of antioxidant-rich herbal infusions and other plant-based nutraceutical products [21].

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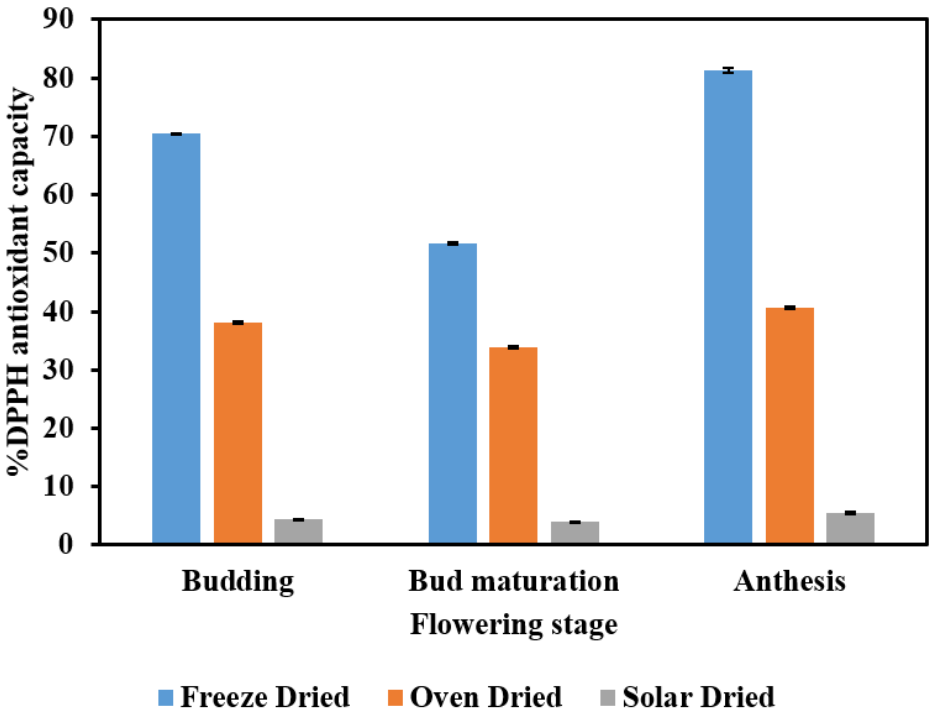


Figure 4: Effect of drying methods and flowering stages on DPPH radical scavenging activity at 10 mg/mL of OS flowers

Differences among drying treatments suggest that antioxidant capacity is influenced not only by concentration but also by structural integrity and chemical composition of phytochemicals [22]. Overall, freeze-dried anthesis flowers demonstrated the highest antioxidant potential, highlighting their suitability for functional ingredient applications.

Effect of drying methods and flowering stages on pigment compounds (betalains and anthocyanins)

The effects of drying methods and flowering stages on betalain and anthocyanin contents are presented in Figure 5 and Figure 6, respectively. Total betalain content (TBC) and total anthocyanin content (TAC) were significantly ($p < 0.05$) influenced by flowering stage and drying method. There was also a significant ($p < 0.05$) interaction between the two treatments, although they exhibited contrasting developmental trends.

Betalain content ranged from 21.29 to 358.1 mg/100 g DW, with the highest value recorded in freeze-dried anthesis flowers and the lowest in solar-dried budding flowers, Figure 5. In contrast, anthocyanin content ranged from 1033 to 2577 mg/100 g DW, with the highest value observed in freeze-dried budding flowers and the lowest in solar-dried anthesis flowers, Figure 6.

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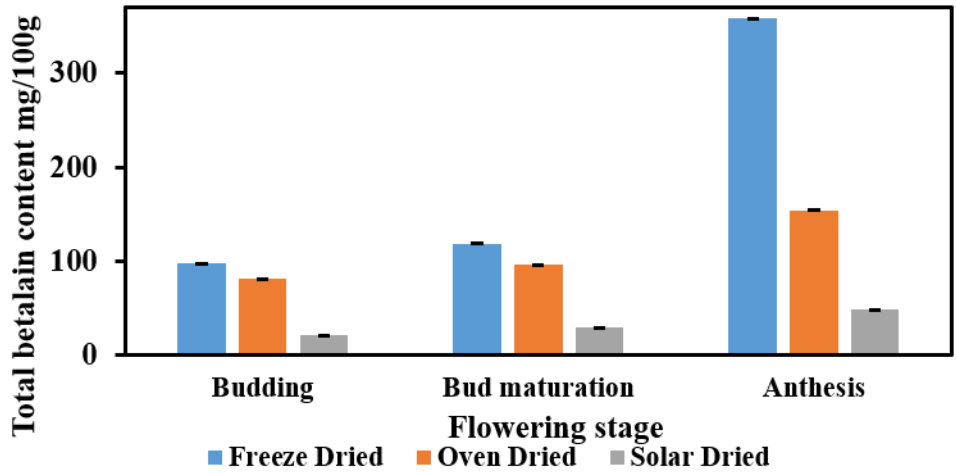


Figure 5: Effect of drying methods and flowering stages on total betalain content of OS flowers

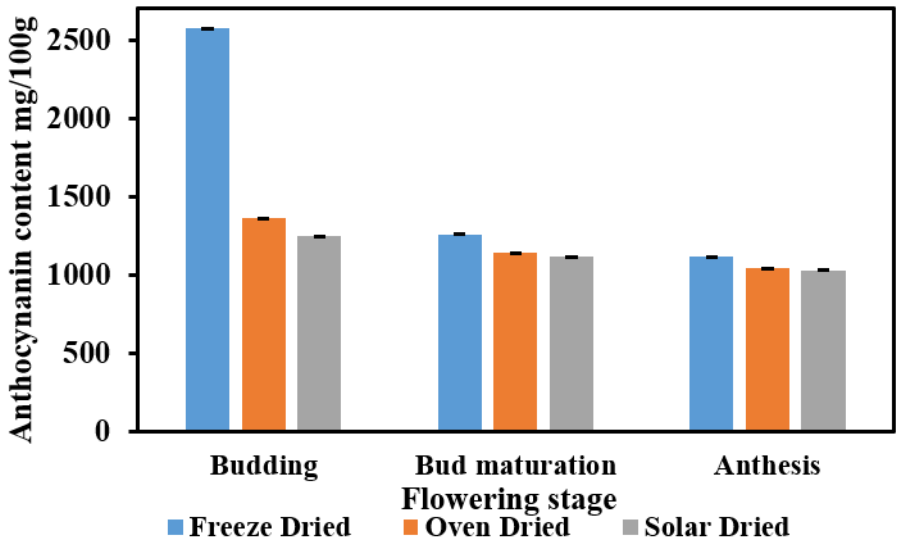


Figure 6: Effect of drying methods and flowering stages on anthocyanin content of OS flowers

Betalains increased progressively from budding to anthesis, indicating enhanced biosynthesis during flower maturation. Their stability was strongly influenced by drying method, with freeze-drying preserving significantly higher levels due to reduced thermal and oxidative degradation. In contrast, oven- and solar-drying promoted pigment breakdown through oxidation and structural disintegration [23]. Other pigment-rich edible flowers have also been found to show similar pigment-retention trends. Freeze-drying was proven to retain higher concentrations of betalains and relevant phenolic compounds in

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Bougainvillea glabra than thermal processing did, while findings on Dahlia pinnata indicated that its petals are a rich source of natural pigments with high antioxidant activity [24]. On a broader scale, freeze-drying is considered one of the most efficient preservation methods for heat-sensitive betalains since it reduces thermal degradation, oxidation and structural destruction during dehydration. These findings support the present results and demonstrate that freeze-drying is a successful method by which the functional qualities and natural color of food and beverage applications of OS flowers are preserved [25].

Anthocyanins displayed an opposite trend, decreasing from budding to anthesis, suggesting higher accumulation in early developmental stages where they function in photoprotection and stress defense [26]. Their strong sensitivity to heat, light and pH explains the marked losses observed under thermal and solar-drying [27]. The contrasting behaviour of betalains and anthocyanins highlights compound-specific regulation during flower development, indicating a shift in dominant pigment systems as maturation progresses. This has important implications for targeted extraction and processing strategies depending on the desired functional pigment. Anthocyanin decreases from budding to anthesis is described in a number of flower species, wherein pigment accumulation is favoured during early developmental stages followed by degradation or reduced synthesis at flower opening. For instance, in Brunfelsia calycina, purple buds decrease in anthocyanin content at anthesis as pigment is degraded more, leading to the development of whiteness [28]. This example offers a general illustration of developmental changes in flower pigmentation, rather than providing direct evidence for the mechanisms regulating anthocyanin content in OS. Although a decrease in anthocyanin content was observed across the flowering stages of OS in this study, the specific biochemical or molecular mechanisms responsible were not examined. Consequently, the observed decline cannot be conclusively attributed to reduced anthocyanin biosynthesis, pigment instability, or increased degradation based on the current findings. Further research investigating the relevant biosynthetic and degradation pathways is required to clarify the mechanisms underlying anthocyanin variation during OS flower development.

Sensory evaluation of anthesis cactus flower with citric acid pretreatment

The sensory evaluation results of anthesis OS flower infusions subjected to citric acid pretreatment are presented in Table 1. Citric acid concentration significantly influenced aroma, taste, mouthfeel, aftertaste and overall liking, whereas appearance was not significantly affected ($p = 0.568$), indicating that visual quality remained stable across treatments. Appearance scores showed no significant differences among treatments (3.69–3.78; $p > 0.05$). In contrast, all flavour-related attributes exhibited statistically significant variation, with aroma ($p < 0.001$), taste ($p < 0.001$), mouthfeel ($p = 0.017$), aftertaste ($p < 0.001$) and overall liking ($p < 0.001$) all improving with citric acid addition.

Citric acid had a pronounced positive effect on the taste, aroma, mouthfeel, aftertaste and general acceptability of infusions from OS flower anthesis, where both 0.5% and 1%

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concentrations of citric acid were superior to the control treatment, whereas appearance was not affected. No significant differences between 0.5% and 1% levels were noted for most sensory properties, implying that low acidification is enough for improving flavor balance, aroma perception, mouthfeel, aftertaste and acceptability without affecting appearance [29]. Preliminary sensory screening revealed that 2% citric acid produced an unacceptable amount of sourness (data not shown), justifying the choice of 0.5-1% level of citric acid as the optimum.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

In this study, it was shown that the flower maturation stage and drying process have a significant effect on the composition of bioactive substances, antioxidant properties and sensory characteristics of OS flowers. Flowers at the stage of anthesis contained the highest amounts of total phenols, total flavonoids, betalains and DPPH radical scavenging activity, whereas the anthocyanin content was maximal at the budding stage. Among all drying processes used in the experiment, freeze-drying gave the best retention of bioactive substances and antioxidant properties of dried Opuntia flowers. However, a 60°C drying process in an oven is more realistic for the production of high-quality functional beverages. In turn, solar-drying showed a loss of bioactive substances and was not the best variant for obtaining high-quality functional ingredients. Pretreatment with citric acid improved the sensory characteristics of the flower infusion from the anthesis stage, the use of 1% citric acid gave the maximal value of acceptability among other tested concentrations, although it was not significantly different from the 0.5% treatment.

It can be concluded that OS flowers are promising sources of natural antioxidants and functional beverage ingredients. In practice, for collecting flowers for functional beverages, it is recommended to harvest the flower at the anthesis stage, use oven-drying and apply pretreatment with citric acid.

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Table 1: Sensory evaluation scores of anthesis OS flower infusions subjected to different citric acid pretreatments

Sample	Appearance	Aroma	Taste	Mouthfeel	Aftertaste	Overall liking
0% citric acid	3.69 ± 0.85[a]	3.30± 1.1[a]	2.89± 1.0[a]	3.44± 1.08[a]	3.24± 1.08[a]	3.36±0.91[a]
0.5% citric acid	3.69 ± 0.91[a]	3.47±0.93[b]	3.39± 1.0[b]	3.67± 0.97[b]	3.42± 1.0[b]	3.51± 0.93[b]
1% citric acid	3.78 ± 0.96[a]	3.71± 0.9[b]	3.61± 1.07[b]	3.72± 0.98[b]	3.68± 1.05[b]	3.76± 0.99[b]
P value	0.57	<0.001	<0.001	0.017	<0.001	<0.001

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